

Appl. No. 10/723,247
Amdt. dated November 15, 2006
Reply to Office Action mailed May 15, 2006

Amendments to the Specification:

Amend the paragraph beginning on page 5, line 25, and ending on page 6, line 9, as follows:

Suitable EPACs include proteins and peptides that comprise one or more phosphorylation sites. As noted above, virtually all protein kinases phosphorylate serine, threonine and/or tyrosine residues, and over 1000 phosphorylation sites comprising serine, threonine and/or tyrosine residues are now known. See, *e.g.*, Kreegipuu et al., "PhosphoBase, a database of phosphorylation sites: release 2.0," *Nucleic Acids Res.*, **27(1)**:237-239 (1999) and <http://www.cbs.dtu.dk/databases/PhosphoBase/>. The proteins and peptides may be naturally-occurring proteins and peptides, fragments of such proteins and peptides and synthetic proteins and peptides (including mutated and partially synthetic forms of naturally-occurring proteins and peptides and wholly synthetic proteins and peptides) that comprise at least one phosphorylatable amino acid. The proteins and peptides must be of a sufficient size (typically greater than about 20 amino acids in length) and/or have a charge (*i.e.*, be hydrophilic) that causes them to remain extracellular.

Amend the paragraph beginning on page 8, line 27, and ending on page 9, line 11, as follows:

Other suitable proteins for use in the practice of the invention include caseins. Whole casein, α -casein, one of the several isoforms of α -casein, β -casein, γ -casein and/or κ -casein and/or fragments of any of the foregoing can be used. Caseins have multiple phosphorylation sites. For instance, β -casein has five phosphorylation sites. Whole caseins, α -caseins, isoforms of α casein, β -caseins, γ -caseins, and κ -caseins are available commercially (from, *e.g.*, Sigma-Aldrich) or can made be prepared by methods well known in the art (see, *e.g.*, U.S. Patents Nos. 5,068,118, 5,739,407, 5,795,611, 5,942,274 and 6,232,094 and <http://www.worthington-biochem.com/CASA>). Preferred are caseins made by recombinant DNA techniques in bacteria, since they will not be phosphorylated. Also, as noted above, α_{s1} casein from human colostrum is naturally unphosphorylated and is, therefore, very convenient to use. It is available commercially

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from Sigma-Aldrich. Dephosphorylation of caseins, if necessary, can be performed as described above. See, *e.g.*, U.S. Patents Nos. 6,355,297 and 5,068,118.

Amend the paragraph beginning on page 12, line 5, and ending on page 12, line 18, as follows:

Since kinases are highly promiscuous, it is anticipated that even random sequences comprising one or more serine, threonine and/or tyrosine residues will be phosphorylated. However, in a preferred embodiment, the synthetic protein or peptide will comprise one or more known phosphorylation sites. As noted above, over 1000 phosphorylation sites comprising serine, threonine and/or tyrosine residues are now known. See, *e.g.*, Kreegipuu et al., "PhosphoBase, a database of phosphorylation sites: release 2.0," *Nucleic Acids Res.*, **27(1)**:237-239 (1999) and <http://www.cbs.dtu.dk/databases/PhosphoBase/>. See also, Aitken, *Mol. Biotechnol.*, **12**:241-53 (1999). Also, methods have been developed for identifying phosphorylatable peptides from random peptide libraries. See Wu et al., *Biochemistry*, **13**:14825-14833 (1994) and Songyang et al., *Curr. Biol.*, **4**:973-982 (1994). Since the EPACs act nonspecifically, the identity of the phosphorylation sites is not critical. When a plurality of phosphorylation sites is used, each phosphorylation site may be the same or different than the other phosphorylation site(s).

Amend the paragraph beginning on page 14, line 21, and ending on page 15, line 6, as follows:

Since kinases are highly promiscuous, it is anticipated that even random sequences comprising one or more serine, threonine and/or tyrosine residues will be phosphorylated. However, in a preferred embodiment, the synthetic peptide will comprise one or more known phosphorylation sites. As noted above, over 1000 phosphorylation sites comprising serine, threonine and/or tyrosine residues are now known. See, *e.g.*, Kreegipuu et al., "PhosphoBase, a database of phosphorylation sites: release 2.0," *Nucleic Acids Res.*, **27(1)**:237-239 (1999) and

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<http://www.cbs.dtu.dk/databases/PhosphoBase/>. See also, Aitken, *Mol. Biotechnol.*, **12**:241-53 (1999). Also, methods have been developed for identifying phosphorylatable peptides from random peptide libraries. See Wu et al., *Biochemistry*, **13**:14825-14833 (1994) and Songyang et al., *Curr. Biol.*, **4**:973-982 (1994). Since the IPACs act nonspecifically, the identity of the phosphorylation sites is not critical. When a plurality of phosphorylation sites is used, each phosphorylation site may be the same or different than the other phosphorylation site(s).

Amend the paragraph beginning on page 64, line 19, and ending on page 65, line 17, as follows:

Cell culture supernatants were analyzed for IL-8 content by enzyme-linked immunosorbent assay (ELISA) ELISA. The IL-8 ELISA was performed as follows. Anti-human IL-8 antibody (Pierce Endogen, Rockford, IL; catalogue number M801-E, lot number CK41959) was diluted to 1 µg/ml in phosphate buffered saline, pH 7.2-7.4, and 100 µl of the diluted antibody was added to each well of Nunc Maxisorb ELISA strip plates. The plates were incubated overnight at room temperature. The liquid was aspirated from the wells, and the plates were blotted on a paper towel. Then, 200 µl of assay buffer (phosphate buffered saline, pH 7.2-7.4, containing 4% BSA) were added to each well, and the plates were incubated for 1 hour at room temperature. The liquid was aspirated from the wells, and the wells were washed 3 times with wash buffer (50 mM Tris, 0.2% Tween-20, pH 7.9-8.1) and were then blotted on a paper towel. Standards and supernatant samples (50 µl/well; standards were diluted in assay buffer) were added to the wells, and the plates were incubated for 1 hour at room temperature with gentle shaking. The liquid was aspirated, the wells were washed 3 times with wash buffer, and the plates were then blotted on a paper towel. Then, 100 µl of biotin-labeled anti-human IL-8 (Pierce Endogen, Rockford, IL; catalogue number M802-E, lot number CE49513), diluted to 60 ng/ml in assay buffer, were added to each well. The plates were incubated for 1 hour at room temperature, the liquid was aspirated, the wells were washed 3 times with wash buffer, and the plates were blotted on a paper towel. Then, 100 µl of HRP-conjugated streptavidin (Pierce Endogen, Rockford, IL; catalogue number

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N100) in assay buffer, were added to each well. The plates were incubated for 30 minutes at room temperature, the liquid was aspirated, the wells were washed 3 times with wash buffer, and the plates were blotted on a paper towel. Finally, 100 μ l of TMB substrate solution (Pierce Endogen, Rockford, IL; catalogue number N301) were added to each well. The plates were incubated for 30 minutes at room temperature. The reaction was stopped by adding 100 μ l/well of 0.18 M H_2SO_4 . The optical densities at 450 nm and 530 nm were read on an ELISA plate reader and the difference (OD 450 – OD 530) calculated.